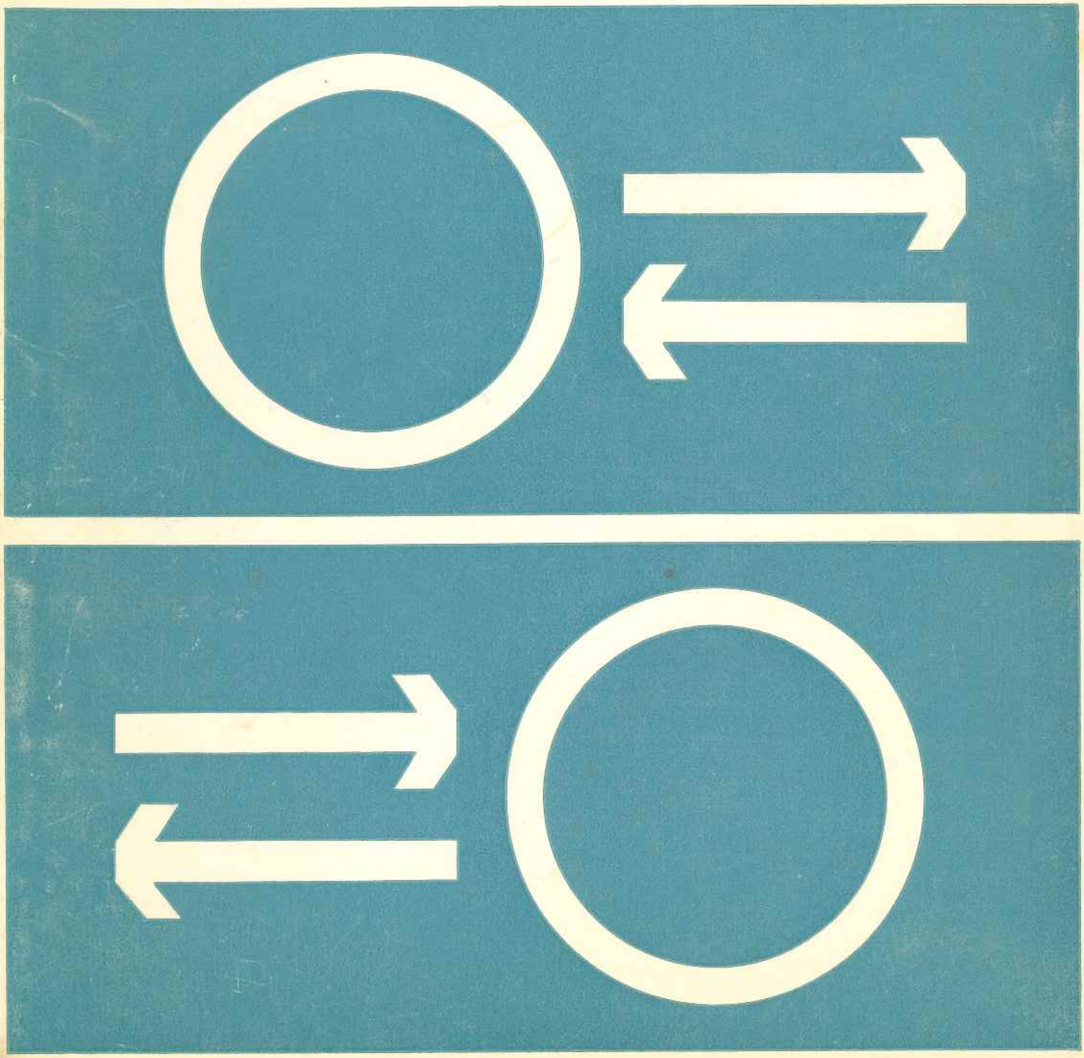
Sephadex® ion exchangers

A guide to ion exchange chromatography



This booklet is intended as an introduction to ion exchange chromatography on Sephadex ion exchangers. Special emphasis has been put on the choice of experimental conditions and working procedures in order to make the booklet useful not only as a reference text but also as a laboratory handbook.

Pharmacia Fine Chemicals will be pleased to answer specific questions concerning Sephadex ion exchangers. Personal communications or reprints of published work describing new applications or techniques are most welcome.

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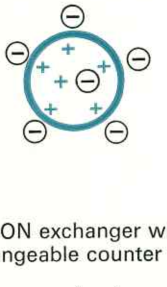
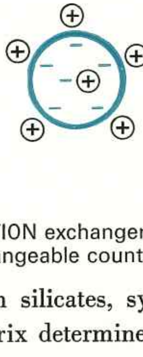
ION EXCHANGE CHROMATOGRAPHY

An ion exchanger is an insoluble material containing chemically bound charged groups and mobile counter ions. The counter ion may be reversibly exchanged with other ions of the same charge without any changes of the insoluble matrix.

If the matrix carries positive groups the counter ions will be negative. Such an ion exchanger will exchange negative ions and is therefore termed an **anion** exchanger. In the same way, if the matrix carries negative groups the counter ions will be positive. Since the positive ions are exchangeable the term **cation** exchanger is used.



ANION exchanger with
exchangeable counter ions



CATION exchanger with
exchangeable counter ions

The **matrix** may be based on aluminium silicates, synthetic resins, polysaccharides etc. The nature of the matrix determines the mechanical stability and flow properties of the ion exchanger, its behaviour towards labile biological substances and to a certain extent its capacity. The presence of **charged groups** is a fundamental property of an ion exchanger. The type of group determines the type and strength of the ion exchanger; the total number of groups and their accessibility determine the capacity. Phenolic hydroxyl, carboxyl and sulphonic groups are commonly used to form cation exchangers and aliphatic or aromatic amino groups to form anion exchangers. Sulphonic and quaternary amino groups are used to form **strong** ion exchangers, the other groups result in **weak** ion exchangers.

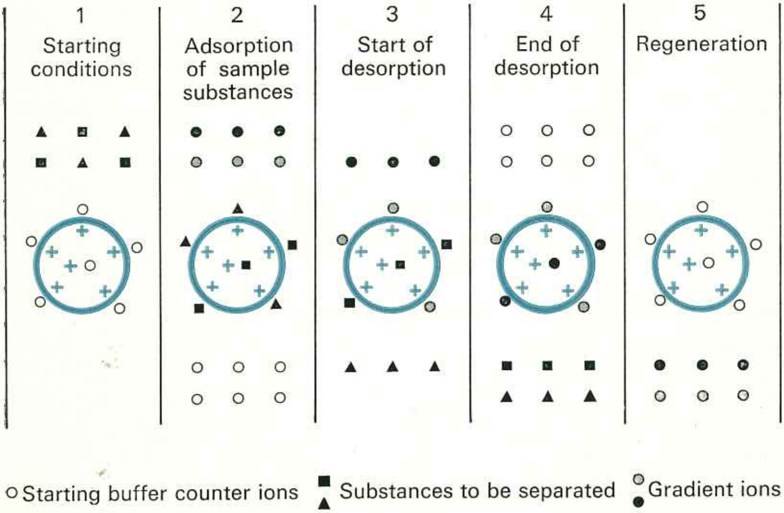
The capacity of an ion exchanger is a quantitative measure of its ability to take up exchangeable counter ions and is therefore of major importance. The capacity may be expressed as total capacity or available capacity.

The **total capacity** is the amount of charged and potentially charged groups per gram dry ion exchanger.

The **available capacity** is the actual capacity obtainable under specified experimental conditions. It is dependent on the accessibility of the functional groups, eluant concentration and ionic strength, the nature of the counter ions and the selectivity of the functional groups towards them. The pH and temperature of the eluant are also important, especially for weakly acidic and weakly basic ion exchangers.

The mechanism by which separation is obtained on an ion exchanger is one of **reversible adsorption.** Most ion exchange experiments are performed in two main stages. These are firstly, the addition and binding of substances to the ion exchanger and secondly the removal of these substances one at a time, separated from each other. Separation is possible since substances normally have different electrical properties and are released from the ion exchangers by changes in salt concentration, pH etc. The conditions under which substances are bound or free are discussed in detail in the sections dealing with choice of experimental conditions. In addition to the ion exchange effect, other types of binding may occur. These are mainly due to Van der Waals forces and polar interactions.

Ion exchange may be carried out in a column or by a batch procedure. Essentially there is no difference between the methods which are both carried out in definite stages. These are equilibration of the ion exchanger, addition and binding of sample substances, change of conditions to produce selective desorption and regeneration of the ion exchanger. These steps are schematically illustrated below.


1
Starting conditions
2
Adsorption of sample substances
3
Start of desorption
4
End of desorption
5
Regeneration
○ Starting buffer counter ions
■ ▲ Substances to be separated
○ ● Gradient ions

Stage 1 shows the ion exchanger in equilibrium with its counter ions. Sample substances are about to enter the ion exchanger bed. At stage 2 the counter ions have been exchanged for sample substances. After this adsorption a gradient is applied. Desorption of one sample species occurs at stage 3. This substance is exchanged for gradient ions and is therefore eluted from the ion exchanger. At stage 4 the remaining sample substance is exchanged for gradient ions and eluted, after which regeneration may be started. The gradient ions are exchanged for counter ions in stage 5 and the ion exchanger is thus regenerated and ready for re-use.

SEPHADEX ION EXCHANGERS

Structure, functional groups and designations

Sephadex ion exchangers are derived from neutral Sephadex by the introduction of functional groups. Sephadex is a modified dextran. The dextran chains are cross-linked to give a three-dimensional polysaccharide network. The functional groups are attached by ether linkages to the glucose units of the dextran chains.

Sephadex is eminently suitable as an ion exchange matrix. Proteins and other labile biological substances are not denatured by the hydrophilic polysaccharide network. Sephadex exhibits very low non-specific adsorption. High capacities can be obtained since high degrees of substitution do not lead to breakdown of the matrix. The bead form gives the ion exchanger superior flow properties. Sephadex ion exchangers, therefore, combine the advantages of cellulose-based and resin-based ion exchangers and are intended specifically for use in biochemical separations.

Sephadex ion exchangers are prepared from Sephadex G-25 and Sephadex G-50. This leads to two groups of exchangers differing in their porosities. Sephadex G-25 is more highly cross-linked than Sephadex G-50 and has, therefore, a lower porosity. The porosities of the ion exchangers are also dependent on the environmental ionic strength, pH and the nature of the counter ions. The availability of two types of different porosities offers flexibility in the selection of the most suitable ion exchanger for a given separation.

The substitution of four different functional groups into the Sephadex G-25 and G-50 matrices leads to eight ion exchangers — four anion and four cation exchangers. The functional groups are:

DEAE $-C_2H_4N^+(C_2H_5)_2H$
QAE* $-C_2H_4N^+(C_2H_5)_2CH_2CH(OH)CH_3$
CM $-CH_2COO^-$
SP $-C_3H_6SO_3^-$

Table 1.

Types		Description	Functional groups	Counter ion
DEAE- Sephadex	A-25 A-50	Weakly basic anion exchanger	Diethylaminoethyl	Chloride
QAE- Sephadex	A-25 A-50	Strongly basic anion exchanger	Diethyl-(2-hydroxypropyl) aminoethyl	Chloride
CM- Sephadex	C-25 C-50	Weakly acidic cation exchanger	Carboxymethyl	Sodium
SP- Sephadex	C-25 C-50	Strongly acidic cation exchanger	Sulphopropyl	Sodium

The letters A and C are added as suffixes to denote either anion or cation exchanger. They are used in conjunction with the numbers -25 and -50 to designate degree of porosity.
From January 1970 SP-Sephadex replaces the earlier SE-Sephadex. The two types have very similar properties.

Sephadex ion exchangers are manufactured to a high standard of purity corresponding to analytical grade. They are tested for proper function and capacity, thus ensuring good reproducibility from batch to batch. Since the ion exchangers are derived from neutral Sephadex types they are all in bead form (the bead diameter for the dry particles is 40—120 μ) and are supplied as dry, free-running powders.

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* (QAE=quaternary aminoethyl)

Properties

Swelling

The swelling properties of Sephadex ion exchangers are related to those of the parent Sephadex types. Thus, the ion exchangers swell in aqueous solvents and the degree of swelling is dependent on the degree of cross-linking — -50 types swell more than -25 types. However, unlike the parent Sephadex types, the degree of swelling varies with the ionic composition of the swelling medium.

Ionic strength dependence. At low ionic strengths, repulsion between the fixed charged groups is maximum and the ion exchangers swell greatly. The degree of swelling decreases with increasing ionic strength because of diminishing repulsion as shown in fig. 1.

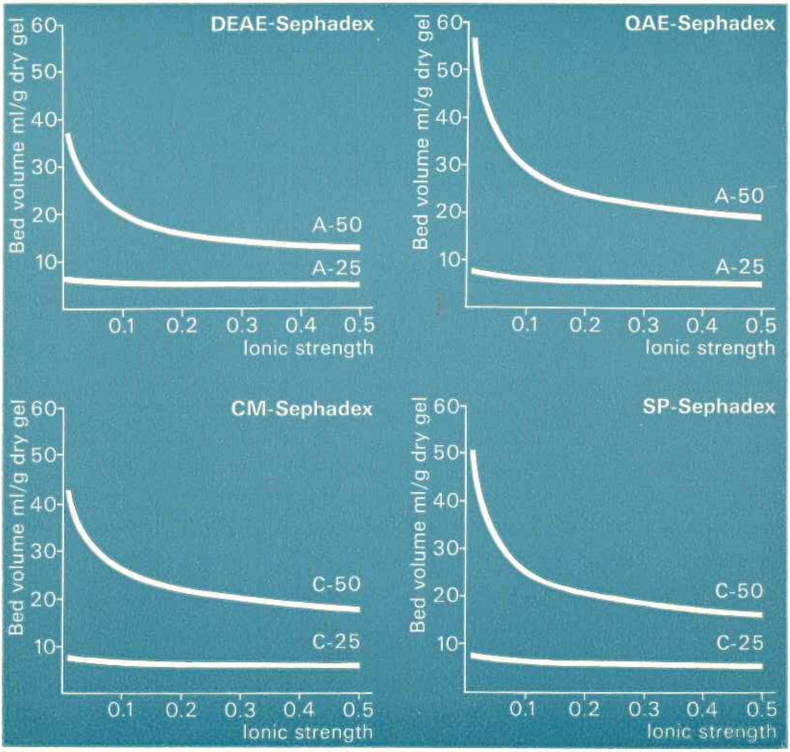

Fig. 1 Bed volumes obtained from 1 gram dry ion exchanger as a function of ionic strength. Bed volumes were determined in a column 1.5×30 cm (K 15/30) at a constant pressure head of 30 cm water. DEAE- and QAE-Sephadex were measured in tris-HCl buffer, pH 7.6, with varying NaCl concentration. CM- and SP-Sephadex were measured in acetate buffer, pH 4.3, with varying salt concentration.

pH dependence. The extent to which an ion exchanger is charged is dependent on pH. Repulsion between charged groups is a maximum at pH values where the ion exchanger is fully charged and decreases as the charged groups become neutralized. This variation in repulsion results in a pH dependent swelling. Fig. 2 gives the bed volumes obtained from 1 gram of dry ion exchanger at varying pH. Note that QAE-Sephadex and SP-Sephadex are fully charged over a very wide pH range and have swelling properties independent of pH.

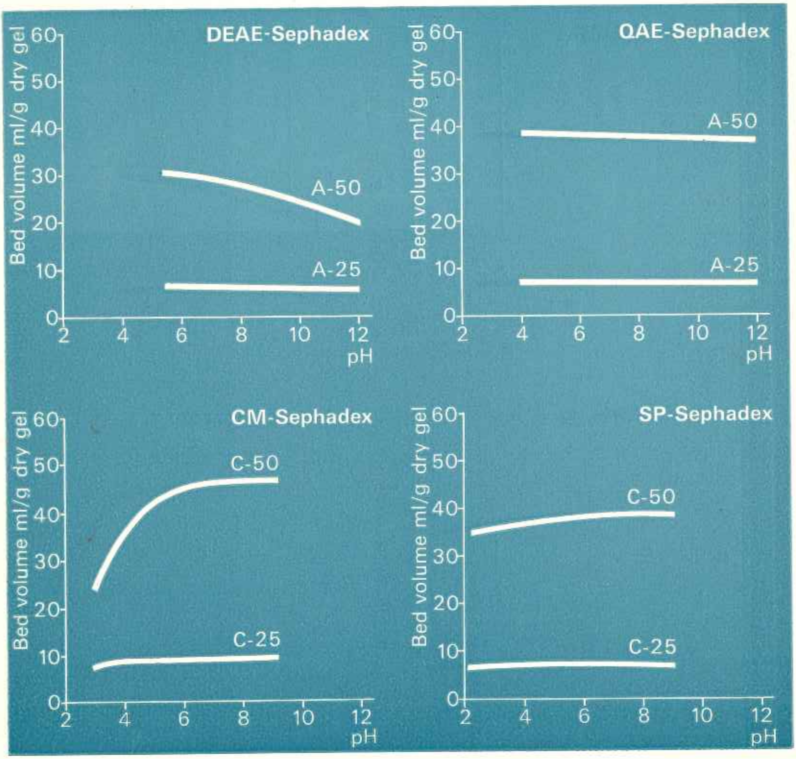

Fig. 2 Bed volumes obtained from 1 gram dry ion exchanger as a function of pH. Bed volumes were determined in a column 1.5×30 cm (K 15/30) at a constant pressure head of 30 cm water. DEAE- and QAE-Sephadex were measured in imidazole and ethylenediamine buffers of varying pH at a constant ionic strength of 0.05. CM- and SP-Sephadex were measured in phosphate buffers of varying pH at a constant ionic strength of 0.05.

Capacity

The difference between "total capacity" and "available capacity" has already been mentioned. Capacity data for Sephadex ion exchangers is given in table 2.

Table 2

Ion exchanger		Total capacity meq/g	Haemoglobin capacity* g/g
DEAE-	A-25	3.5 ± 0.5	0.5
Sephadex	A-50		5
QAE-	A-25	3.0 ± 0.4	0.3
Sephadex	A-50		6
CM-	C-25	4.5 ± 0.5	0.4
Sephadex	C-50		9
SP-	C-25	2.3 ± 0.3	0.2
Sephadex	C-50		7

* The haemoglobin capacity is the amount of haemoglobin (MW 69,000) reversibly bound by one gram (dry weight) of ion exchanger. This value was measured for DEAE- and QAE-Sephadex in tris-HCl buffer pH 8.0, $I=0.01$, and for CM- and SP-Sephadex in acetate buffer pH 5.0, $I=0.01$.

The available capacity can be calculated either per gram of dry ion exchanger or per ml bed volume. In both cases Sephadex ion exchangers have higher capacities than other comparable ion exchangers. Sephadex ion exchangers are, by virtue of their high capacity at high ionic strength, particularly suitable for work with proteins, since the low ionic strength region where aggregation and insolubility of proteins may occur, can be avoided.

Porosity dependence. The available capacity varies with the accessibility of the functional groups, i.e. the porosity of the beads and the size of the molecule. Ion exchangers prepared from Sephadex G-50 are more porous than those prepared from G-25 and thus, for molecules which are able to penetrate the -50 types more than the -25 type ion exchanger, the -50 type has a higher available capacity. The haemoglobin capacity given in table 2 is, therefore, only a guide to the protein capacity.

Ionic strength dependence. Ions in the vicinity of the charged groups on the ion exchanger compete for these groups. An increase in ionic strength, therefore, increases the competition for the binding sites on the exchanger thereby decreasing interaction between sample substances and the charged groups and leading to a reduced capacity.

pH dependence. The capacity is dependent on the number of charged groups and may, therefore, vary with pH. The titration curves for the various ion exchangers are shown in fig. 3.

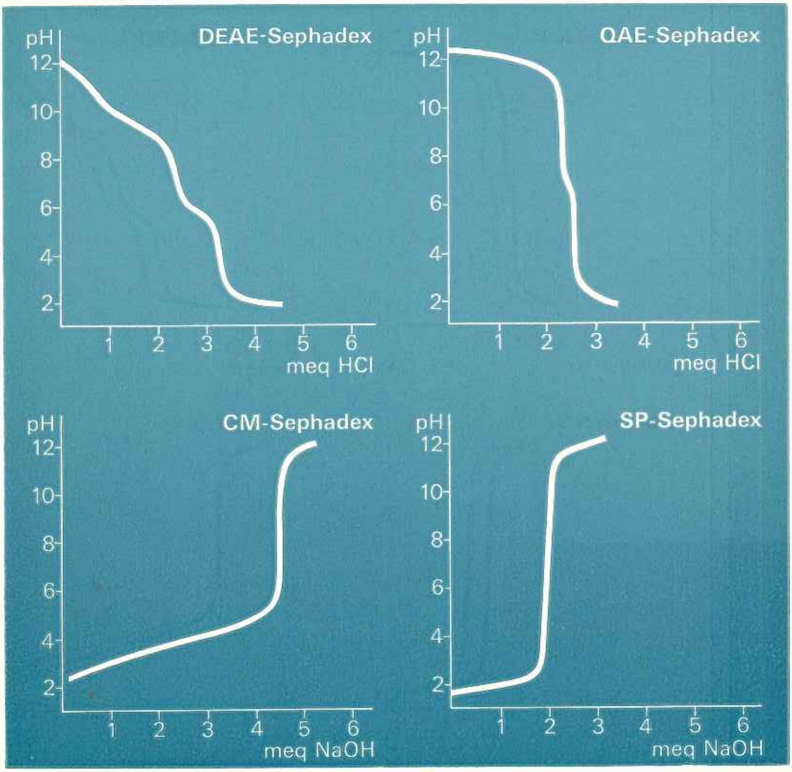

Fig. 3 Titration of 1 gram ion exchanger in 1M KCl.

From the titration curves it can be seen that the capacities of the strong ion exchangers QAE- and SP-Sephadex are constant over a very wide pH range. The titration curve for DEAE-Sephadex reveals that more than one type of charged group is present. This is due to side reactions which occur during the introduction of DEAE-groups into the Sephadex matrix. The normal DEAE-groups have pK 9.5 and thus below pH 9 DEAE-Sephadex has excellent capacity for proteins.

Stability

Sephadex ion exchangers are insoluble in all solvents. They are stable in water, salt solutions, organic solvents, alkaline and weakly acidic solutions. In strongly acidic solutions, hydrolysis of the glucosidic linkages may occur. Thus pH values below about 2 should be avoided particularly at elevated temperatures. Treatment with strong oxidising agents and with solutions containing dextranase should also be avoided. During regeneration, the ion exchanger may be exposed for a short time to 0.1 M NaOH or 0.2 M HCl without appreciable hydrolysis.

Sephadex ion exchangers can be sterilized in the wet state by autoclaving for up to 30 minutes at 120°C at pH 7. During autoclaving, minute quantities of carbohydrate material are released: these can be washed away with sterile buffer.

Prevention of bacterial and microbial growth is dealt with in a separate section (see page 35).

EXPERIMENTAL TECHNIQUE

Choice of ion exchanger

Functional groups

Choice according to charge. Substances are bound to ion exchangers if they have a charge opposite to that of the ion exchanger: the binding is electrostatic and is reversible. The correct choice of ion exchanger, as regards functional group, depends, therefore, on the **net charge** of the substances to be chromatographed.

Some substances carry only one type of charged group. The choice of anion or cation exchanger is then clear-cut. Substances which carry both negatively and positively charged groups are amphoteric and their net charge is thus dependent on pH. At low pH the net charge is positive, at high pH it is negative. At the point of zero net charge, the **isoelectric point,** the substances are not bound to any type of ion exchanger.

Proteins are amphoteric polyelectrolytes and can normally be bound to both anion and cation exchangers. There is, however, a limiting factor, namely the stability of the protein. In the following discussion the "range of stability" refers to the pH range in which the protein does not undergo denaturation. The net charge of a protein as a function of pH is shown in fig. 4. From the figure it can be seen that below the isoelectric point the protein has a net positive charge and is therefore adsorbed by cation exchangers. Above the isoelectric point the protein can be attached to anion exchangers since it carries a net negative charge. In this case the choice of ion exchanger is determined largely by the range of stability of the protein.

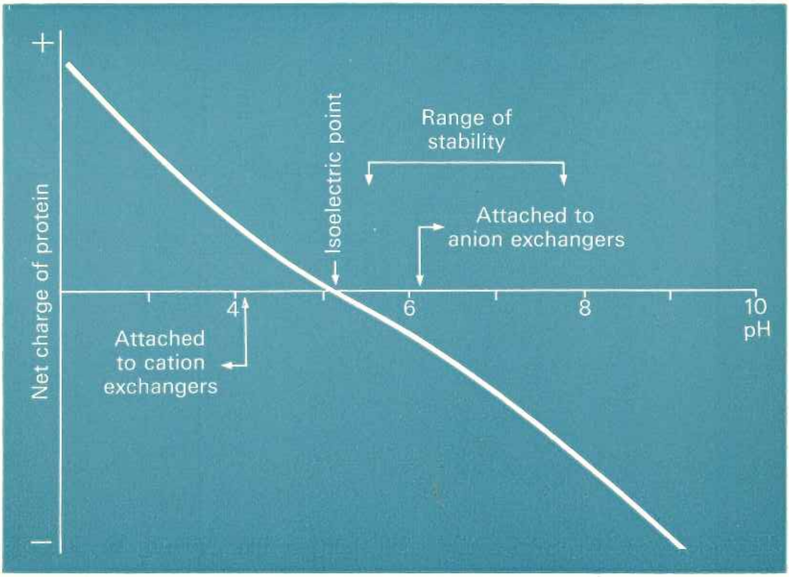

Fig. 4 The net charge of a protein as a function of pH. The pH ranges in which the protein is bound to anion or cation exchangers and an arbitrary range of stability are shown.

It is possible therefore to draw the following guide lines for the choice of ion exchanger.

- If the protein is not subject to denaturation at pH above its isoelectric point it should be chromatographed on either of the **anion** exchangers DEAE- or QAE-Sephadex.
- If the protein is not subject to denaturation at pH below its isoelectric point it should be chromatographed on either of the **cation** exchangers CM- or SP-Sephadex.
- If the range of stability of the protein reaches 1 pH unit above and below the isoelectric point it may be chromatographed on either anion or cation exchangers.

Very often the isoelectric point of the protein is unknown. In such cases a simple test can be performed to determine which type of ion exchanger can be used.

Materials

Anion exchanger, DEAE- or QAE-Sephadex A-50
Cation exchanger, CM- or SP-Sephadex C-50
Buffer solutions
Spectrophotometer

Method

The range of stability of the protein is not normally known if the isoelectric point is unknown. In this case choose a buffer pH of about 8 for the anion exchanger test and a buffer pH of about 5 for the cation exchanger test.

Weigh out 0.05 gram of each ion exchanger into separate test tubes. Add about 30 ml of the appropriate buffer (ionic strength 0.1) to each ion exchanger. Equilibrate by decantation with at least two more aliquots of buffer. Allow the ion exchanger to sediment and remove supernatant until the total volume (ion exchanger plus supernatant) is 10 ml. Add equal amounts of protein solution to each ion exchanger. Shake the suspension to ensure that all the protein comes into contact with the ion exchanger. Again, allow the ion exchanger to sediment. Prepare a standard solution of the protein and measure the extinction. Remove the supernatant above each ion exchanger and measure the extinctions. If several proteins are present, measurement of extinction may not be a suitable method since proteins may exist in the supernatant and may also be bound to the ion exchanger. In such cases, determination of enzyme activity etc. may be necessary.

Results and conclusion

Compare the extinctions (or concentrations) of the protein in the supernatant from each ion exchanger with the standard. From this comparison it should be clear which ion exchanger has taken up protein and therefore which is more suitable for chromatographic experiments.

Influence of other factors. When choosing the correct ion exchanger group, factors other than the net charge on the substances must be taken into account.

In a mixture of substances carrying known net charges it is possible to choose the ion exchanger so that **labile** or **stable, interesting** or **uninteresting** substances are bound. In the case of labile substances, it is very often better to choose an ion exchanger which binds the more stable substance and thus avoid denaturation which may occur during the actual ion exchange.

Binding to the ion exchanger is also dependent on the **amount of charge** carried by the substances. The greater the charge, the greater is the binding. The **ionic strength** of the solution has a pronounced effect on the binding. An increase in ionic strength causes increased competition for the binding sites on the ion exchanger; the electrostatic properties of the system are also changed, decreasing the electrostatic interaction between the ion exchanger and the counter ions. These effects, which together reduce the strength with which substances are bound, are discussed under both "Choice of buffer" and "Elution".

The choice of weak or strong ion exchanger depends on the effect of pH on the charge and lability of substances to be separated. The **strong** anion exchanger, QAE-Sephadex, is especially useful when weakly acidic substances, which require high pH for ionization, are to be chromatographed. Similarily, SP-Sephadex can be used in a very low pH range. The strong ion exchangers may, however, not be suitable for chromatography of extremely labile compounds: in such cases the **weak** ion exchangers DEAE- and CM-Sephadex are recommended.

Porosity type

The porosity of the ion exchanger does not influence the binding mechanism but influences the capacity since charged groups on the inner surface of the ion exchanger may not be available to very large molecules.

The ion exchangers prepared from Sephadex G-25 (designated A-25 and C-25) are intended primarily for chromatography of peptides, small proteins and other low molecular weight solutes which are able to penetrate the ion exchanger beads. Ion exchangers based on the Sephadex G-50 matrix (designated A-50 and C-50) are more suitable for substances excluded by the -25 types of exchangers. Substances of very high molecular weight can be chromatographed on either type of ion exchanger since only functional groups on the surface of the beads will be available to them. In such cases the -25 types of ion exchangers are preferable since the charge density (density of functional groups) is greater on the surface of the less swollen beads. The fact that the -25 types swell less than the -50 types also gives the -25 types of exchangers greater rigidity and therefore better flow properties. Table 3 gives an approximate guide to the choice of ion exchanger type based on the molecular weight of the substance to be separated.

Table 3

Molecular weight of protein		Preferred exchanger
Low MW	$<30,000$	A-25, C-25
Medium MW	30,000—200,000	A-50, C-50
High MW	$>200,000$	A-25, C-25

When choosing the ion exchanger according to molecular weight it is unnecessary to consider the possibility of gel filtration effects. It has been suggested that separations on Sephadex ion exchangers depend on both ionic interactions and differences in molecular size of the various solutes. Although steric factors are always involved, they only affect the separation of charged solutes by determining the available capacity for such substances. Only uncharged solutes can be fractionated according to size as in gel filtration, and then it should be noted that the fractionation range of the ion exchanger will not correspond to the parent Sephadex type (i.e. G-25 or G-50).

Choice of buffer

Choice of buffer substance. If the buffering ions carry a charge opposite to that of the functional groups of the ion exchanger, they will take part in the ion exchange process and cause local disturbances of the pH equilibrium. It is possible, therefore, to formulate two simple rules.

- When possible, **cationic** buffers should be used with **anion** exchangers.
- When possible, **anionic** buffers should be used with **cation** exchangers.

Some examples are given in table 4.

Table 4

Ion exchanger	Buffer
anion DEAE-Sephadex QAE-Sephadex	**cationic** Alkyl amines, aminoethyl alcohol, ammonia, barbital (below pH 7.5), ethylene diamine, imidazole, pyridine, tris
cation CM-Sephadex SP-Sephadex	**anionic** Acetate, barbital, citrate, glycine, phosphate

Choice of buffer pH and ionic strength. The buffer pH and ionic strength are normally chosen so that the substances of interest are bound to the ion exchanger. Starting conditions should, however, not be too far removed from the point at which substances are released from the ion exchanger.

The buffer pH should be chosen so that substances have a net charge opposite to that of the ion exchanger. The starting pH should be about 1 pH unit above the isoelectric point with anion exchangers and about 1 pH unit below the isoelectric point with cation exchangers.

Substances begin to dissociate from the ion exchanger about 0.5 pH units from their isoelectric points at ionic strength 0.1, (*Lampson* and *Tytell*, 760).* The buffer pH should also be chosen so that the functional groups are fully charged. Limits are also set by the chemical stability of the ion exchangers. Table 5 gives the pH ranges within which Sephadex ion exchangers should be used.

Table 5

Ion exchanger	Recommended pH range
DEAE-Sephadex	2— 9
QAE-Sephadex	2—10
CM-Sephadex	6—10
SP-Sephadex	2—10

The buffer ionic strength influences the binding and therefore the capacity for different substances. A major advantage of Sephadex ion exchangers is that the initial ionic strength of the buffer may be high. Even at fairly high ionic strength, Sephadex ion exchangers have high capacity. Furthermore, the risk of protein denaturation and the problems involved with buffering capacity and control of pH are avoided. An additional factor is that the swelling dependence of Sephadex ion exchangers is more marked at low than at high ionic strength. Problems of swelling and contraction are thus minimized by choosing high ionic strength. A well accepted starting ionic strength is 0.1.

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* Number refers to the Literature Reference List

Preparation of the ion exchanger

To obtain reproducible results it is essential that equilibrium is established between the ion exchanger and the eluant. An advantage of Sephadex ion exchangers is that they do not require precycling, i.e. treatment with acid and alkali before equilibration. Ion exchangers should be swollen at the pH to be used in the experiment. Complete swelling occurs in 1—2 days at room temperature or after 2 hours (at neutral pH) on a boiling water bath. Decantation of fines is normally unnecessary. Vigorous stirring should be avoided in order not to damage the beads. Sephadex ion exchangers are supplied in their salt forms and therefore the preparation procedure depends on whether or not the counter ions are to be changed.

Preparation of DEAE- and QAE-Sephadex with chloride counter ion and CM- and SP-Sephadex with sodium counter ion. Stir the required amount of ion exchanger into an excess of starting buffer. The starting buffer must contain the same ion as that originally present in the ion exchanger. Remove the supernatant and replace with fresh buffer several times during the swelling period. Instead of decantation, the ion exchanger can be washed extensively on a Büchner funnel after the initial swelling.
Example: if SP-Sephadex is to be used in acetate buffer it is swollen directly in a solution containing sodium acetate and acetic acid thus preserving sodium as the counter ion. Note that the buffering acetate ion is not bound by the ion exchanger.

Preparation of ion exchangers with counter ions other than chloride or sodium. Stir the required amount of ion exchanger into an excess of 0.5—1.0 M solution of the salt of the new counter ion. Allow the ion exchanger to swell completely in this solution. After sedimentation and decantation resuspend the ion exchanger in the buffer to be used in the experiment. Repeat several times.
Example: if ammonium acetate is to be used with QAE-Sephadex the ion exchanger is swollen first in a concentrated sodium or ammonium acetate solution. The chloride ions are thus exchanged for acetate ions. The QAE-Sephadex is then equilibrated several times in the ammonium acetate buffer to be used.

The amount of ion exchanger required for a given experiment depends on the amount of substance to be chromatographed and can be determined by a simple test. Choice of sample size and column size are interdependent. The required amount of ion exchanger depends on its available capacity for the sample substances and no more than 10—20 % of this capacity should be used.

Materials

Ion exchanger (choice of anion or cation exchanger is described on page 17)
Buffer solution
Spectrophotometer

Method

Weigh 0.05 g (A-50, C-50 types) or 0.25 g (A-25, C-25 types) of ion exchanger into a test tube. Equilibrate by decantation with approximately 30 ml buffer. Adjust the volume of ion exchanger plus supernatant to 10 ml and add a known amount of sample substance either in buffer or as dry solid. Shake carefully and allow the ion exchanger to sediment. Prepare a standard solution of the sample substance and measure its extinction. Separate the ion exchanger from the supernatant by filtration and measure the extinction of the filtrate. (See also experiment on page 17).

Results and conclusion

Calculate the amount of substance bound by the ion exchanger and thus the amount required to bind the sample to be used in the chromatographic experiment. Multiply this by a factor of 5 to 10 to obtain the amount of ion exchanger required for chromatography. The figure obtained can be used for both column and batch procedures.

Column chromatography

Choice of column

Column height. Normally the conditions are such that the sample substances are adsorbed within the upper 1—2 cm of the ion exchanger bed. A bed height of 20 cm is often sufficient. Longer beds may be necessary for resolution of very complex mixtures.

Column diameter. If the bed height is fixed, the column diameter can be calculated on the basis of how much ion exchanger is to be packed in the column. Column diameter is, therefore, dependent on the required capacity of the ion exchanger bed.

Column construction. The material used in the construction of the column should be chosen to prevent destruction of labile biological substances. The bed support should be designed so that clogging by biological materials or gel particles does not occur. A fine mesh nylon net is the best solution to the problem. Dead spaces must be kept at a minimum so that separated substances are not remixed. The range of columns available from Pharmacia Fine Chemicals have been especially designed for gel filtration and ion exchange chromatography and incorporate all the features mentioned above. In addition the Gel and Eluant Reservoirs which are available for each column type make packing a simple, one-step procedure.

Packing the column

Mix the swollen ion exchanger with starting buffer so that it can be easily poured. If the slurry is too thick, air bubbles will be trapped in the gel when it is poured. Packing from a thin suspension, however, introduces problems of convection currents arising during packing. Set up the column and check that it is mounted vertically. Ensure that the bed support is covered by about 1 cm of eluant, that no air remains in or under the bed support and that the column outlet is closed. Pour the ion exchanger into the column either down a glass rod or down the side of the chromatographic tube thus avoiding bubble formation. The use of a Gel and Eluant Reservoir enables all the ion exchanger suspension to be poured at one time. Allow the gel to stabilize for 5—10 minutes. Fill the column with starting buffer and connect to a buffer reservoir.

Adjust the column outlet so that the operating pressure is about 1 cm H_2O/ cm height of gel bed. Open the column outlet and allow the ion exchanger to pack under a constant pressure head. When packing is completed, layer about 0.5 cm Sephadex G-25 Coarse (swollen in the same buffer as the ion exchanger) onto the top of the ion exchanger bed to act as bed surface protection. This is most easily done by applying the Sephadex suspension onto the ion exchanger using a pipette. Do not remove excess buffer from above the packed bed while carrying out this operation.

Checking the packing. Irregularities in the ion exchanger bed can normally be seen in transmitted light from a lamp held behind the column. If irregularities or air bubbles appear the bed must be re-packed.

Equilibrating the column

Before the actual experiment is run the ion exchanger must be allowed to reach ionic equilibrium with the starting buffer. Run at least two bed volumes of buffer through the ion exchanger bed. This not only allows the system to reach equilibrium but also stabilizes the bed. In principle, counter ion concentration, conductivity and pH of the eluant should be checked against the ingoing solution. In practice, it is normally sufficient to measure the pH of the effluent.

Sample

Sample concentration. The amount of sample substances which can be applied to a column is dependent on the available capacity of the column. Either the column size or the amount of sample must be decided first. The amount of sample can be determined together with the amount of ion exchanger as described on page 23.

Sample composition. The ionic composition should be the same as that of the starting buffer. If it is not, it can be changed by gel filtration on Sephadex G-25, dialysis or possibly by dilution with starting buffer.

Sample volume. If the ion exchanger is to be developed with the starting buffer, the sample volume is important and must be determined in conjunction with the amount of sample. If, however, the ion exchanger is to be developed with a gradient, starting conditions are chosen so that all important substances are adsorbed at the top of the bed. In this case, the actual amount of sample substances applied is of far greater importance than the sample volume. For example, if a mixture of haemoglobins is to be separated on SP-Sephadex C-50 at least 0.7 gram haemoglobin per gram (dry weight) ion exchanger can be chromatographed. 0.7 gram is 10 % of the haemoglobin capacity (g/g) of the ion exchanger. Whether 0.7 gram of haemoglobin are applied, dissolved in 2 ml or 50 ml, is unimportant since all the protein will be taken up in the top of the bed. In practice, this means that large volumes of dilute solutions — such as pooled fractions from a preceeding gel filtration step — can be applied directly to the ion exchanger without prior concentration.

Sample application. There are two basic methods of sample application.

- Remove excess eluant from above the bed surface either by draining eluant from the column, pipetting off the excess or sucking it off with a syringe. Make sure that the column outlet is closed. Pipette the sample onto the drained surface and open the column outlet. When all the sample has entered the bed, wash the top of the column with small aliquots of starting buffer and connect the column for elution.
- Leave excess eluant on top of the ion exchanger. Attach some very thin capillary tubing to a syringe and flare the free end by heating it gently. Fill the syringe with sample and layer it on top of the bed by arranging the end of the tubing just above the surface and slowly pressing out the sample. Note that the sample must be denser than the eluant or made denser by the addition of, for instance, glucose. Connect the column for elution.

If gradients are to be used, leave about 2 cm of buffer above the ion exchanger bed. This will ensure proper mixing of the gradient.

Elution

Ion exchanger, buffer pH and ionic strength have been chosen so that interesting substances are bound to the ion exchanger. The parameters which can be varied, therefore, to cause the fractionation of bound substances are pH and ionic strength. Occasionally conditions can be chosen such that sample components are separated by elution with equilibrating buffer. The column is then said to be developed by **starting condition** procedure.

Change of pH. The net charge on an amphoteric molecule is pH dependent. Change of pH towards the isoelectric point of the substance renders it neutral and therefore causes it to be desorbed and eluted from the ion exchanger.

Change of ionic strength. At low ionic strength, competition for the charged groups on the ion exchanger is at a minimum and substances are bound strongly under these conditions. Increasing the ionic strength increases the competition and thus reduces the interaction between the ion exchanger and the sample substances thus causing their elution.

Continuous gradients and stepwise elution. The components in the sample usually have different affinities for the ion exchanger and either continuous or stepwise changes in pH or ionic strength cause their elution. **Continuous pH gradients** are difficult to produce since simultaneous changes in ionic strength, although small, normally occur. Linear pH gradients are not obtained by mixing buffers of different pH in linear volume ratios since the buffering capacities of the systems produced are pH dependent. **Stepwise pH gradients** are easier to produce and much more recommendable. pH gradients combined with controlled ionic strength changes can be used. **Continuous ionic strength gradients** can be prepared by mixing two buffers of different ionic strength. If the volume ratio is changed linearly the ionic strength is changed linearly. **Stepwise ionic strength gradients** are produced by using buffers of fixed ionic strength. The direction of gradients is summarized in table 6.

Table 6

Ion exchanger	Direction of	
	pH gradient	ionic strength gradient
Anion exchanger	decreasing	increasing
Cation exchanger	increasing	increasing

Stepwise elution is technically simpler but it has disadvantages. Substances released by a change of pH or ionic strength are eluted close together. Peaks have sharp fronts and pronounced tailing which may lead to the appearance of false peaks if a buffer change is introduced too early. Continuous gradients cause substances to be eluted in symmetrical peaks and give better resolution. The total volume of eluant should be about 5 times the bed volume of the ion exchanger. The differences between continuous and stepwise gradient elution are shown in fig. 5.

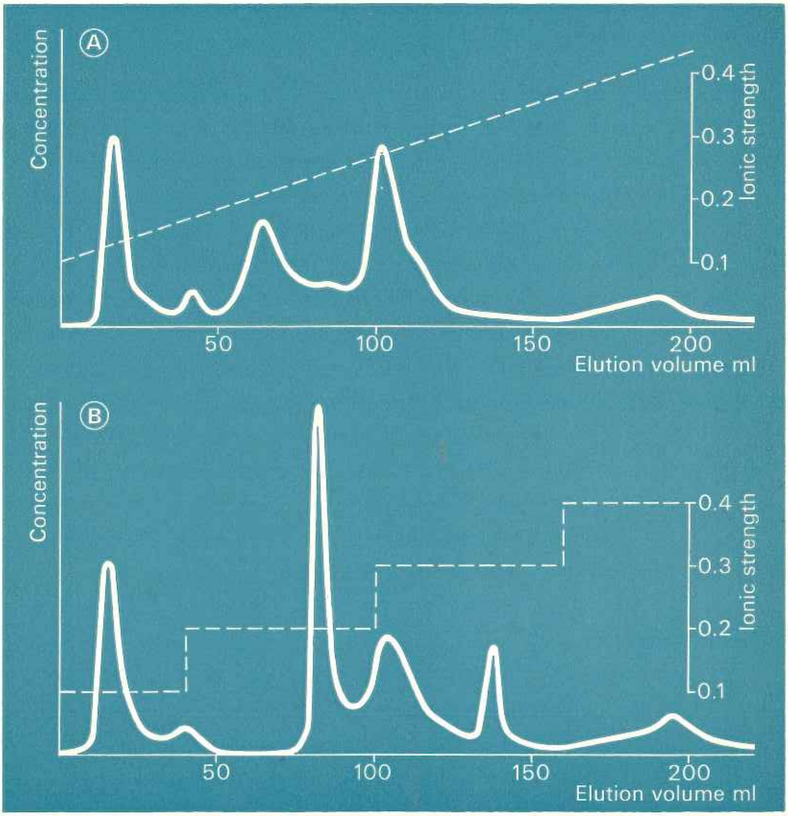


Fig. 5 Continuous gradient and stepwise elution of bovine serum on QAE-Sephadex A-50. Bed dimensions: 1.5×26 cm (column K 15/30). Sample: 4 ml 3 % (w/v) lyophilized bovine serum. Eluant: 0.1 M tris - HCl buffer, pH 6.5. Curve A was obtained using a continuous salt gradient to 0.5 M NaCl. Curve B was obtained using a stepwise NaCl gradient. Flow rate: 0.2 ml/min. The first peak in both diagrams represents IgG. The fourth peak in curve A represents serum albumin. Stepwise elution caused the albumin to be eluted in two peaks (4 and 5). Other peaks were not identified. (From Pharmacia Fine Chemicals, Uppsala, Sweden).

Gradient apparatus. Apparatus to produce continuous gradients are commercially available but simple devices which can be made in any laboratory workshop are shown in fig. 6.

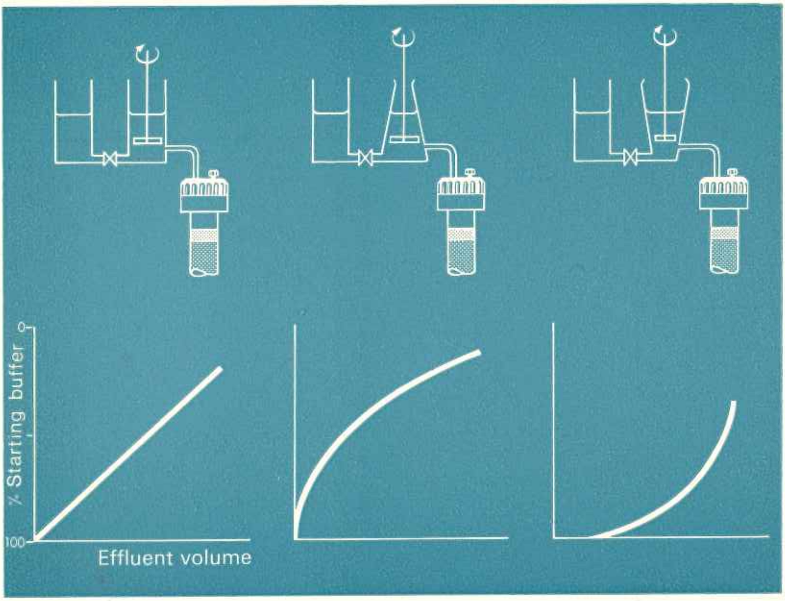


Fig. 6 Gradient apparatus.

If two containers of the same shape are used, the ratio between the buffers changes linearly with the volume withdrawn from the apparatus; by using containers of different shape non-linear gradients can be obtained.

Resolution using a continuous gradient. The resolution depends on the steepness of the gradient and the flow rate. A steep gradient releases substances closer to each other than a shallow one and thus results in poorer resolution. This can be seen in fig. 7 where curve B was obtained with a steeper gradient than curve A. Curve C was obtained with the same gradient as curve A but the flow rate was considerably higher. It is clear that better resolution is obtained at the lower flow rate.

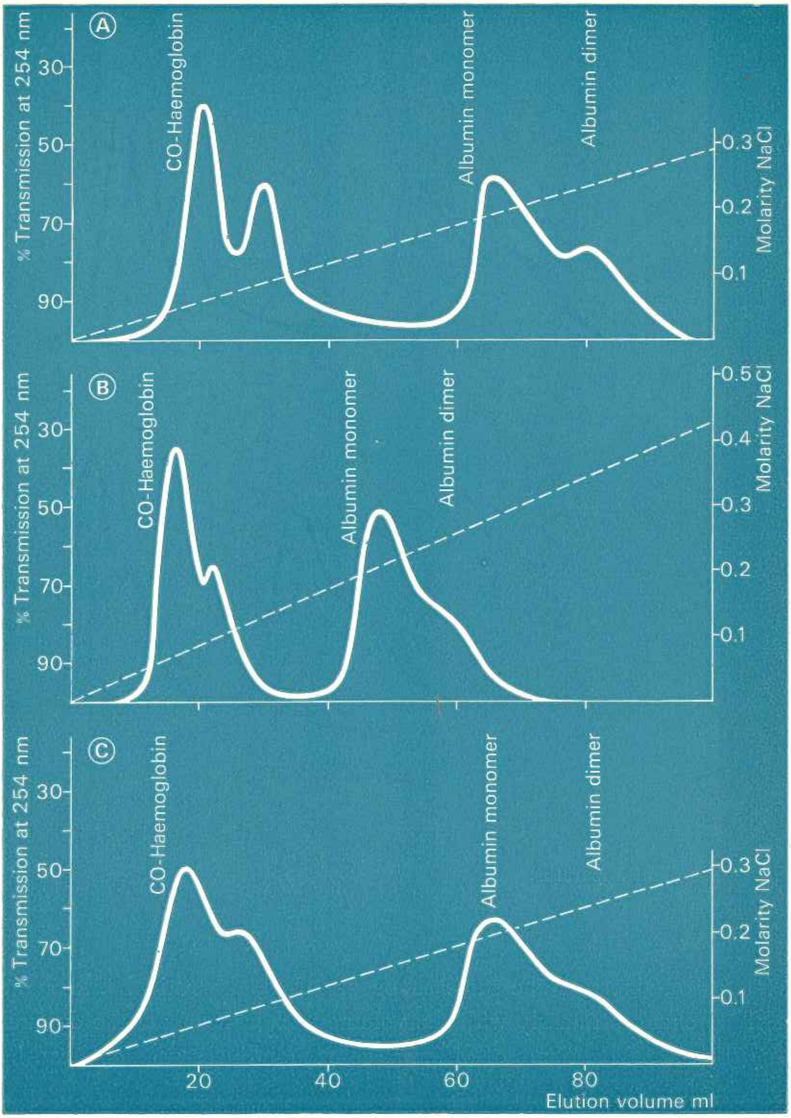


Fig. 7 Separation of haemoglobin and albumin on DEAE-Sephadex A-50, 0.1 M tris-HCl buffer at pH 8.3. The sample consisted of a mixture of O_2-haemoglobin, CO-haemoglobin, albumin monomer and albumin dimer. In cases A and B the flow rate was the same, but the gradients differed in steepness. Cases A and C differed in flow rate. In A the rate of flow was 8 $ml.cm^{-2}.h^{-1}$ and in C 20 $ml.cm^{-2}.h^{-1}$.

(From Pharmacia Fine Chemicals, Uppsala, Sweden.)

Flow rate

Flow rate can be expressed either as a volumetric flow, i.e. ml/h or as a linear flow, i.e. cm/h. Flow expressed in cm/h is the same as the flow in ml per cm^2 cross section area per hour.
The linear flow rate is dependent on the particle size of the bed material, the bed height and diameter, the pressure drop over the bed, the eluant viscosity and other factors.

Particle size dependence. Flow rate increases with increasing particle size. Theoretically a strongly swollen ion exchanger should give high flow rates. However, large, strongly swollen beads are more easily compressible leading to reduced flow rates. In laboratory columns these effects may balance each other. Better flow rates are obtained on the -25 types than on the -50 types of ion exchangers.

Bed height dependence. Flow rate increases with decreasing bed height. In experiments with continuous ionic strength gradients the bed height decreases with increasing salt concentration giving rise to a steadily increasing flow rate. Variations in the flow rate can be eliminated by using a pump instead of gravity feed.

Bed diameter dependence. The linear flow rate decreases with increasing bed diameter although the volumetric flow rate increases.

Viscosity dependence. The flow rate increases with decreasing viscosity. Thus columns run at high temperatures give higher flow rates than those run at low. If viscous samples are applied the overall eluant viscosity is raised only slightly because sample substances become separated, diluted and eluted from the column.

Pressure drop dependence. The dependence of flow rate on the pressure drop is shown in fig. 8. Initially the flow rate increases almost linearly with the operating pressure. The A-25 and C-25 types of ion exchangers are rather rigid gels and maximum flow rates are therefore not used. The A-50 and C-50 types of exchangers, however, behave like the porous, neutral Sephadex G-types with high water regain, i.e. a maximum flow rate is reached at pressures normally used in laboratory experiments. Above this pressure the gel bed becomes more and more compact causing the flow rate to drop.

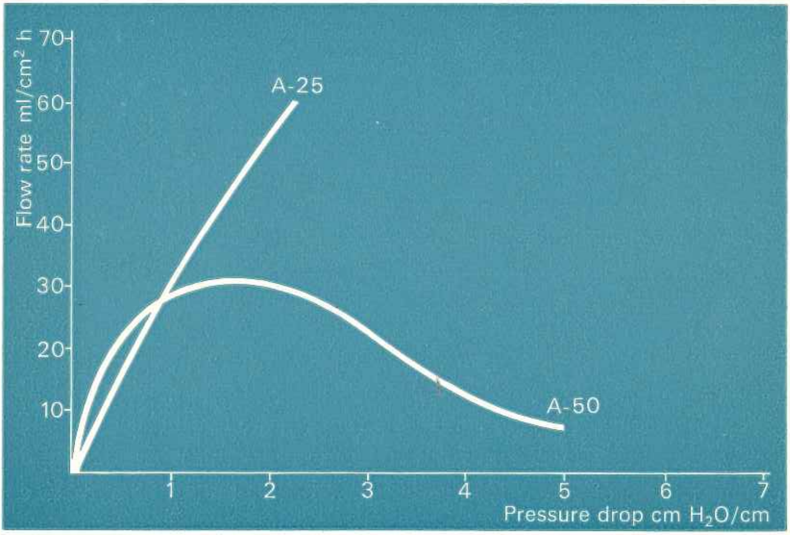


Fig. 8 The flow rate as a function of the pressure drop across a bed of DEAE-Sephadex.
Bed dimensions: 2.5×45 cm. pH: 7. Ionic strength: 0.1.

Other factors. Flow rate is only indirectly dependent on temperature. Increase in temperature generally lowers the eluant viscosity leading to higher flow rates. The flow rate is also dependent on the way in which the bed is packed and the porosity of the packing material. Beds which are tightly packed give lower flow rates than those which are loosely packed. The porosity is a constant of the bed material and for Sephadex ion exchangers it is dependent on the water regain of the material.

Batch separation on Sephadex ion exchangers

There is essentially no difference between a column developed by stepwise elution and a batch procedure. Either the substance of interest or contaminants may be attached to the ion exchanger. Although batch procedures are less efficient than the column techniques, they have certain advantages, especially in manufacturing scale processes. Batch separation is a very rapid technique and no technical difficulties are caused by the swelling or shrinkage of the ion exchanger. The shrinkage may even be an advantage in some applications.

Batch separation is carried out by stirring the ion exchanger, previously equilibrated in the appropriate buffer, with the solution to be treated until the mixture has reached equilibrium. This usually takes about one hour. The slurry is then filtered and the residue washed with the buffer solution. In cases of incomplete adsorption, this procedure should be repeated with a new batch of ion exchanger. The substance on the residual ion exchanger can be desorbed fractionally by resuspending the slurry in buffer solutions of higher ionic strength or modified pH.

Regeneration

It is normally uneconomical to regenerate the small amounts of ion exchanger needed for analytical experiments. Large amounts of exchangers should be regenerated according to the following procedure.

Removal of adsorbed substances and contaminants. Wash with salt solutions of increasing concentration until an ionic strength of about 2 has been reached. The salt should contain the counter ion to the ion exchanger to facilitate equilibration. This should remove any substances bound by ionic forces.

Contaminants such as lipids, proteins etc. can be removed by washing with 0.1 M NaOH followed by distilled water, buffer or salt solution until free from alkali. Lipids may also be removed by washing with alcohol solutions or non-ionic detergents such as Pluronic® F 68.

Regeneration step. Wash with distilled water or if very large quantities of ion exchanger are used with starting buffer or a salt solution containing the counter ion to the ion exchanger. Re-equilibrate with starting buffer. Note that re-equilibration cannot normally be carried out in a column because the gel will swell considerably during this treatment.

Storage

Dry Sephadex ion exchangers are hygroscopic but can be stored for several years without deterioration in tightly closed bottles.
In the wet state, Sephadex ion exchangers are stable for several months provided microbial growth is prevented. DEAE-Sephadex will eventually turn brown when stored in the free amino form. All swollen ion exchangers should be stored in their salt form in buffered suspension containing an antimicrobial agent.

Regenerated ion exchangers, in their salt form, can be dried by the following procedure.
Wash the ion exchanger with very dilute neutral buffer until free of excess salts and other contaminants. Remove excess liquid on a Büchner funnel. Shrink the gel by addition of alcohol solutions of increasing concentration until 96 % alcohol is reached. Ethanol should be used for DEAE-, QAE- and CM-Sephadex and methanol for SP-Sephadex. The gel must be allowed to reach equilibrium at each stage during the shrinking. Finally remove excess alcohol from the gel on a Büchner funnel, wash with ether and leave to dry. Clumps may form during this procedure but they will fall apart during reswelling. A slow shrinking procedure prevents clump formation.

Antimicrobial agents

Bacterial and microbial growth can seriously interfere with the chromatographic properties of Sephadex ion exchangers and may obstruct the flow through the bed. In prolonged experiments and during storage in the swollen state an antimicrobial agent should be added to the ion exchanger. The antimicrobial agent should be chosen so that it is not bound to the ion exchanger. In many instances microbial growth can be prevented by merely removing substrates and phosphates from the ion exchangers.

Antimicrobial agents for anion exchangers

Phenyl mercuric salts, 0.001 %, effective in weakly alkaline solutions.
Hibitane®, (Chlorhexidine) 0.002 %.

Antimicrobial agents for cation exchangers

Sodium azide, 0.02 %.
Merthiolate® (Thimerosal, ethyl mercuric thiosalicylate) 0.005 %, most effective in weakly acidic solution.

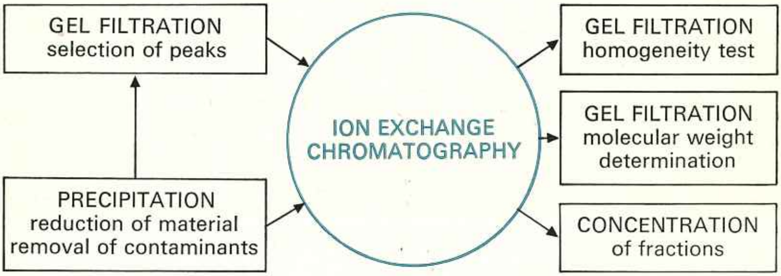
Antimicrobial agents for anion and cation exchangers

Chloretone (trichlorobutanol), 0.05 %, effective only in weakly acidic solutions.
Organic solvents are effective only at high concentrations and are thus of very limited interest.

APPLICATIONS

Sephadex ion exchangers have proved to be invaluable in the separation and purification of labile biological substances. The few examples given in this section have been chosen from the many hundreds of articles published which describe the use of Sephadex ion exchangers in protein separations, enzyme purifications, hormone preparations, nucleic acid and nucleotide studies, carbohydrate separations etc. Information on specific studies can be found in the "Literature Reference List" available separately from Pharmacia Fine Chemicals.

Ion exchange chromatography is seldom used as a technique on its own. In combination with other techniques it is a powerful analytical and preparative tool. Ion exchange chromatography is very often used together with gel filtration. In the case of crude preparations, gel filtration should be carried out first, thereby reducing the number of peaks obtained in the ion exchange step. After ion exchange, however, gel filtration can be used as a check on homogeneity or for molecular weight determination. Precipitation techniques should normally be performed prior to ion exchange since they tend to reduce the amounts of material to quantities more suitable for chromatography. Precipitation also very often removes gross contaminants. Fractions obtained from ion exchange columns often need to be concentrated. This is easily done with Sephadex G-25 or G-50.



The examples given below have been chosen to illustrate the principles discussed in the previous sections and to give the reader an idea of the applications of ion exchange chromatography with Sephadex ion exchangers.

Chromatography of proteins

CM-Sephadex C-50, which has a maximum capacity between pH 6 and 10, has been used by *Dozy* and *Huisman* (2561)* to fractionate different haemoglobin types. These authors used a pH gradient from pH 6.7 to 8.4 to elute haemoglobins F, A, A_2, S and C.

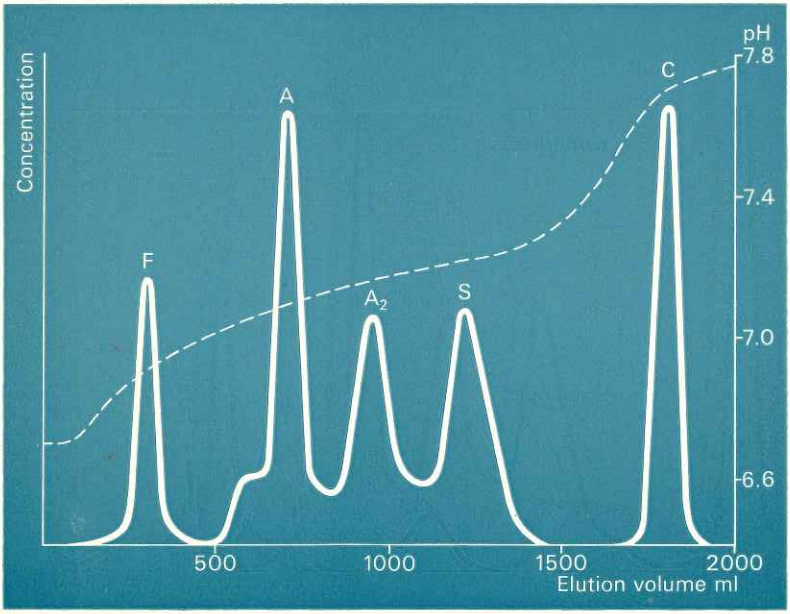


Fig. 9 Elution diagram of an artificial mixture of haemoglobins F, A, A_2, S and C on CM-Sephadex C-50.
Bed dimensions: 0.9×60 cm. Eluant: tris-maleic acid buffers, pH 6.5 to 8.0. Flow rate: 20 ml/h (Dozy, A. M., Huisman, T. H. J., J. Chromatog. 40 (1969) 62—70. Reproduced by kind permission of the Authors and the Publisher.)

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* The numbers in parentheses after references refer to the number of the article in the Literature Reference List.

Wachsmuth and *Pfleiderer* (235) used the anion exchanger DEAE-Sephadex A-50 to fractionate the different LDH isoenzymes. The five isoenzymes were eluted using a continuous NaCl (ionic strength) gradient in an order corresponding to increased migration towards the anode in electrophoresis, i.e. increasing net negative charge.

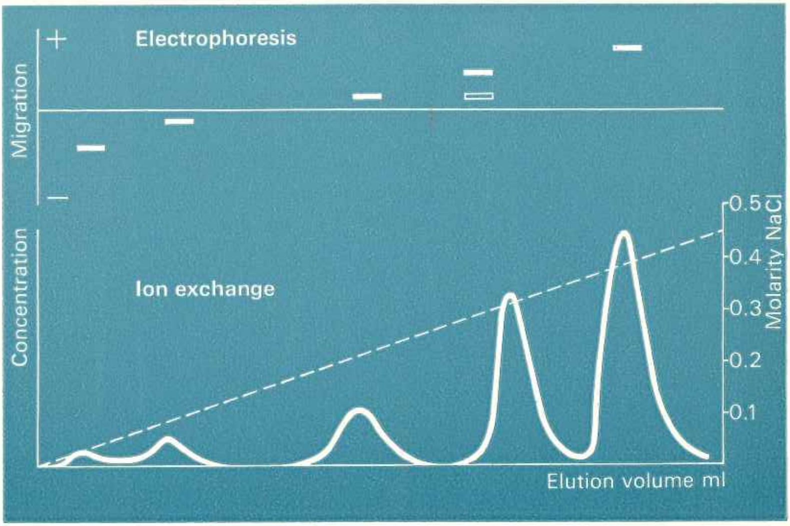

Fig. 10 Elution diagram of lactate dehydrogenase isoenzymes on DEAE-Sephadex A-50.

Bed dimensions: 0.8×100 cm. Eluant: 0,033 M phosphate buffer, pH 7.2, with an increasing salt gradient to 0.5 M NaCl. The upper part of the diagram shows the high voltage electrophoresis pattern obtained in 0.06 M Veronal buffer pH 8.6. (Wachsmuth, E. D., Pfleiderer, G., Biochem. Z. 336 (1963) 545—556. Reproduced by kind permission of the Authors and the Publisher.)

Slegers, Simon et al. (2211), who have previously shown that cobra venom contains mainly basic proteins, accomplished a fractionation of the venom components into 14 fractions on SE-Sephadex C-25 using a continuous ionic strength gradient. This application not only illustrates the fractionation of basic substances on a cation exchanger but also the high resolution obtainable on Sephadex ion exchangers.

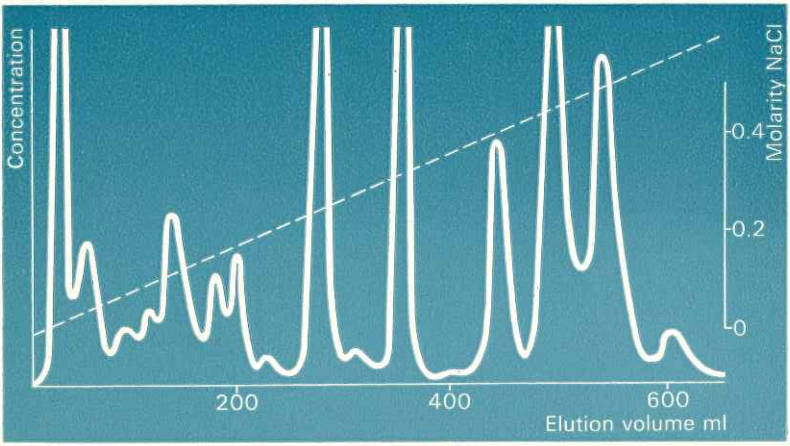

Fig. 11 Elution diagram of Cobra (*Naja naja atra*) venom on SE-Sephadex C-25. Bed dimensions: 1.5×40 cm. Eluant: 0.05 M phosphate buffer, pH 6, with a linear salt gradient to 0.6 M NaCl. Flow rate: 0.5 ml/min. (Slegers, J., Simon, J., et al., J. Chromatog. 36 (1968) 241—243. Reproduced by kind permission of the Authors and the Publisher).

Chromatography of hormones

A combination of gel filtration on Sephadex G-100 and ion exchange chromatography with DEAE-Sephadex A-50 was used by *Peckham* (1367) to purify growth hormones from man and monkey. Gel filtration of a crude simian hormone preparation resulted in two peaks, one of which contained the hormone plus contaminants. This peak when run on DEAE-Sephadex with starting condition development, gave two peaks. Contaminants were eluted first, followed by the purified hormone.

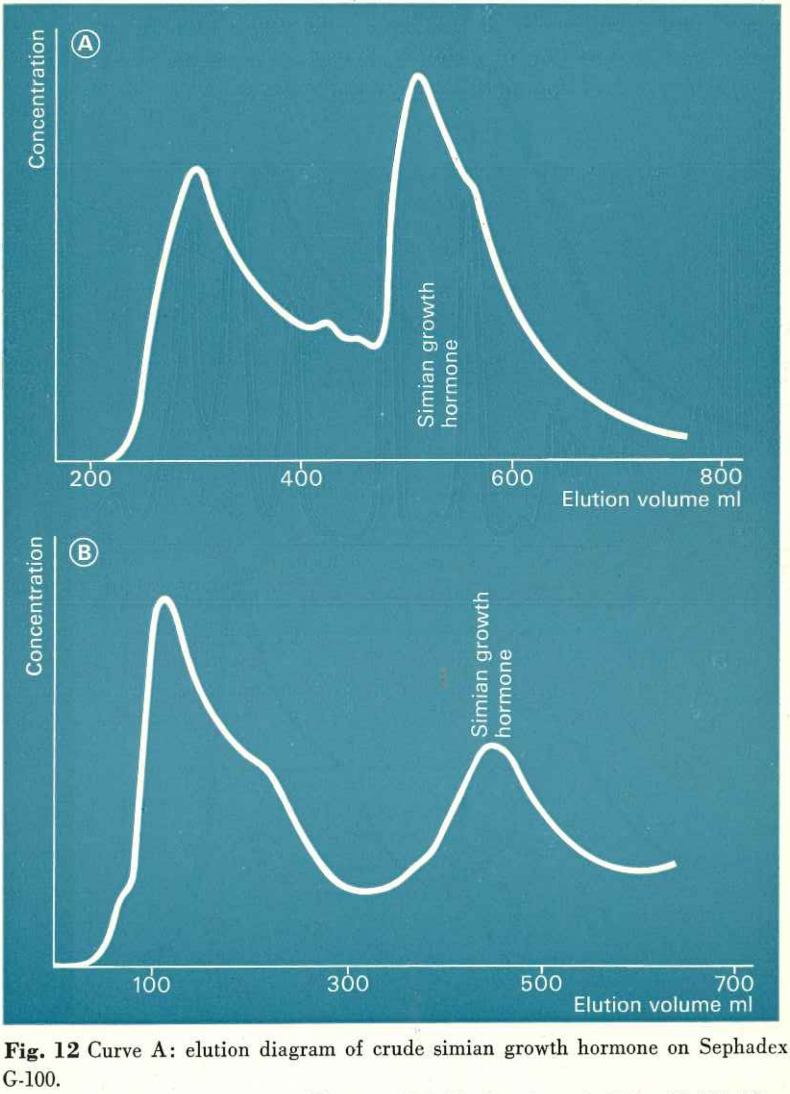

Fig. 12 Curve A: elution diagram of crude simian growth hormone on Sephadex G-100.
Bed dimensions: 2.5×100 cm. Eluant: 0.05 M phosphate buffer, pH 6.8. Flow rate: 10 ml/h.

Curve B: elution diagram of simian growth hormone (obtained by gel filtration on Sephadex G-100) on DEAE-Sephadex A-50.
Bed dimensions: 3.2×40 cm. Eluant: 0.5 M phosphate buffer, pH 6.8. Flow rate: 10 ml/h. (Peckham, W. D., J. Biol. Chem. 242 (1967) 190—196. Reproduced by kind permission of the Author and the Publisher).

Hähnel (880) used DEAE-Sephadex A-25 for the separation of free oestrogens from conjugated oestrogens in urine with a stepwise sodium chloride gradient. Furthermore some oestrogen conjugates could be separated from each other. Quantitative separation of oestrogens from most of the urinary pigments and non-oestrogenic chromogens was obtained.

Chromatography of nucleic acids and nucleotides

Sephadex ion exchangers have been used extensively for nucleic acid separations especially for the preparation of transfer ribonucleic acids. Thus *Nishimura, Harada* et al. (1597), *Seno* and *Kobayashi* (2446) and many others have prepared tRNAs from different sources with different specificities.

Caldwell (2835) chose DEAE-Sephadex A-25 for chromatography of tissue nucleotides since it has a high exchange capacity (3.5 ± 0.5 meq/g) and exhibits minimal non-specific interaction with naturally occurring and synthetic purine and pyrimidine derivatives. The author has also pointed out the advantage of using a bead form ion exchanger, i.e. uniformly packed columns are easily prepared. Extracts of Ehrlich ascites carcinoma cells were exposed to different ^{14}C labelled amino acids and the nucleotides identified after chromatography on DEAE-Sephadex. The elution pattern obtained with a mouse liver extract is shown in fig. 13.

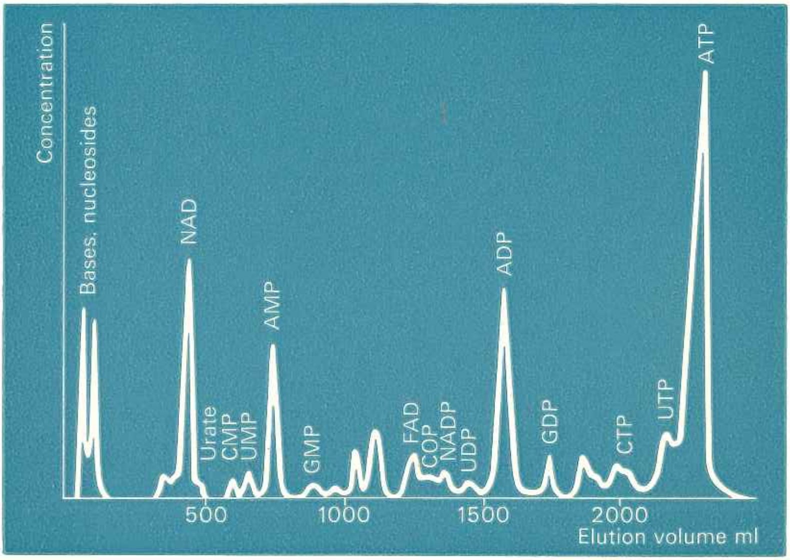

Fig. 13 Elution diagram of tissue nucleotides in an extract of mouse liver on DEAE-Sephadex A-25.

Bed dimensions: 1.0×90 cm. Eluant: Triethylammonium acetate buffer, pH. 4.7, of increasing molarity (concave concentration gradient). Flow rate: 50 ml/h. (Caldwell, I. C., J. Chromatog. 44 (1969) 331—341. Reproduced by kind permission of the Author and the Publisher).

The advantages of the A-25 and C-25 types of ion exchangers for chromatography of low molecular weight solutes are illustrated by the separation of adenosine phosphates on DEAE-Sephadex A-25 and the separation of mononucleotides on QAE-Sephadex A-25 (from the Research Dept., Pharmacia Fine Chemicals). The use of QAE-Sephadex at high pH is also illustrated by this example.

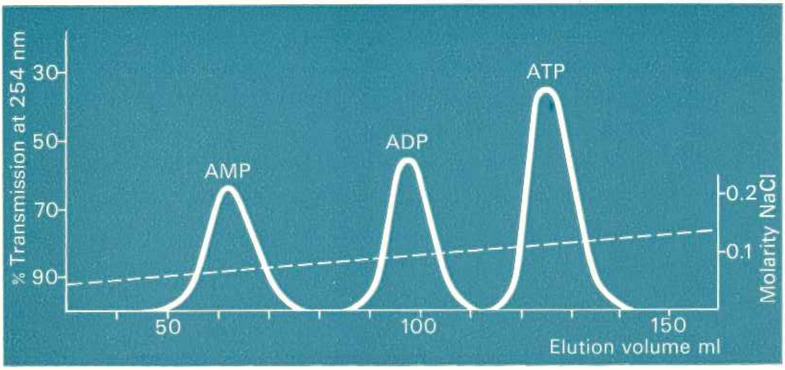

Fig. 14 Separation of the adenosine phosphates on DEAE-Sephadex A-25.
Eluant: 0.1 M tris-HCl buffer, pH 8.3.
(From Pharmacia Fine Chemicals, Uppsala, Sweden.)

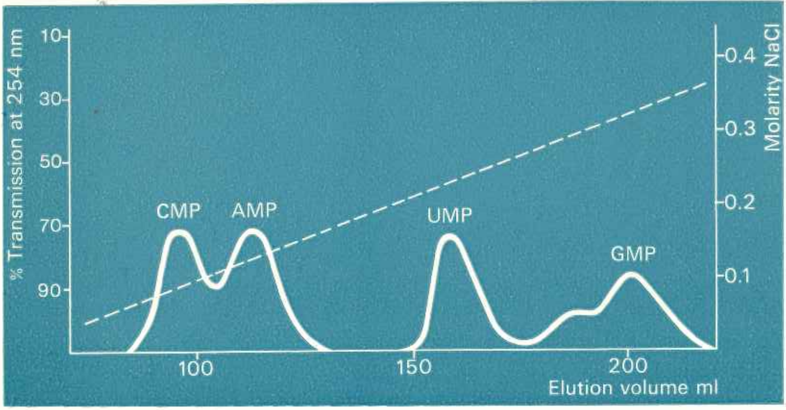

Fig. 15 Separation of mononucleotides on QAE-Sephadex A-25.
Eluant: sodium carbonate-bicarbonate buffer, pH 9.7, ionic strength 0.02.
(From Pharmacia Fine Chemicals, Uppsala, Sweden.)

Chromatography of carbohydrates

Conjugated carbohydrates often carry charged groups permitting their separation on ion exchangers. The literature contains many references to the separation of mucopolysaccharides, sialopolysaccharides, pneumococcal polysaccharides etc. on Sephadex ion exchangers.

As an example *Barker, Hayes* et al. (1735) fractionated soil polysaccharides (of bacterial origin) on DEAE-Sephadex A-50 using a continuous ionic strength gradient although stepwise elution was found more convenient on a large scale.

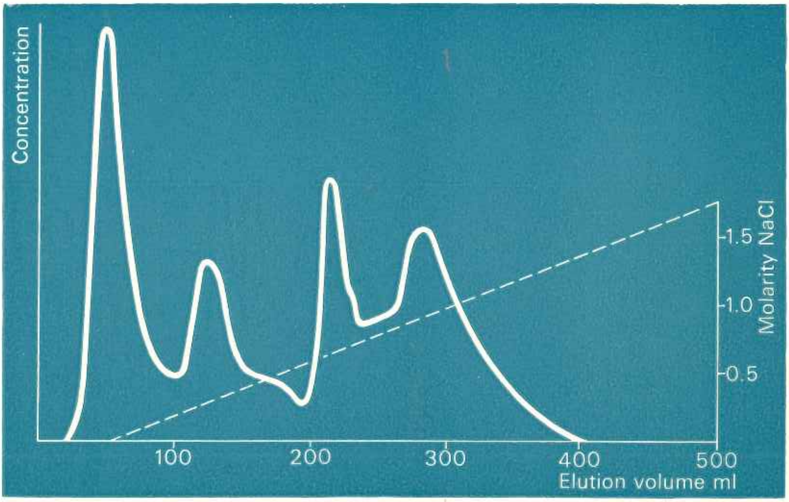

Fig. 16 Elution diagram of a high molecular weight soil polysaccharide on DEAE-Sephadex A-50.

Bed dimensions: 3.4×45 cm. Eluant: phosphate buffer, pH 6, with a linear salt gradient to 2 M NaCl. (Barker, S. A., Hayes, M. H. B. et al., Carbohydrate Res. 5 (1967) 13—24. Reproduced by kind permission of the Authors and the Publisher).

Batch procedures

Björling (229) took advantage of the high capacity of DEAE-Sephadex and its swelling dependence on ionic strength in the purification of ceruloplasmin from a Cohn fraction. In small scale column experiments he found that in acetate buffer at ionic strength 0.15 and pH 6.8 only ceruloplasmin was bound to the gel whilst other proteins passed straight through the column. The procedure could then be developed into a large scale batch procedure.

Ceruloplasmin enriched paste was dissolved in acetate buffer and contacted with DEAE-Sephadex under the conditions given above. The supernatant was filtered off and the ion exchanger washed with buffer until the filtrate was protein free. Solid NaCl was then added causing the gel to shrink. The buffer released by this contraction had an ionic strength of 0.30 and contained ceruloplasmin. Ceruloplasmin was not only purified but also concentrated by this procedure.

In the ceruloplasmin purification, the important substance was bound to the ion exhanger. Purification can, however, be carried out by binding the contaminants and allowing only the important substance to remain in solution. This method has been used by *Baumstark, Laffin* et al. (526) and *Perper, Okimoto* et al. (1673) for the purification of IgG. Undialyzed serum is mixed with DEAE-Sephadex equilibrated with 0.01 M phosphate buffer, pH 6.5. Under these conditions all the serum proteins except IgG are bound by the ion exchanger and the IgG can be recovered from the filtrate. After regeneration the ion exchanger may be immediately re-used.

Production applications

Sephadex ion exchangers have high capacities and are, therefore, highly suitable for large scale preparative and production applications. If column ion exchange is to be carried out on a production scale, swelling and shrinkage of the ion exchanger need to be minimized and "in-column" regeneration developed. With the strong ion exchangers QAE- and SP-Sephadex, this is possible if substances can be eluted by pH changes at constant ionic strength. Such conditions are the basis of a method developed at Pharmacia Fine Chemicals for the purification of IgG (*Joustra* and *Lundgren*, 17th Annual Colloquium, Protides of the Biological Fluids, Brugge, Belgium, 1969).

QAE-Sephadex A-50 is packed and equilibrated in ethylene diamine — acetate buffer, pH 7.0, $I=0.1$. Serum is diluted, brought to the same ionic strength as the buffer and applied to the column. When the column is developed with the equilibrating buffer only IgG is eluted. After IgG elution, the pH is changed to 4 (acetate buffer, ionic strength 0.1) and the residual serum proteins eluted. No changes in the bed volume occur during this procedure. The bed is then re-equilibrated with starting buffer and a new sample applied. If the bed becomes contaminated it can be washed clean with a non-ionic detergent solution.

AVAILABILITY AND ORDERS

Sephadex ion exchangers are available through all subsidiaries and representatives as listed on the inside back cover. If there is no representative in your country please order directly from PHARMACIA FINE CHEMICALS AB, UPPSALA, SWEDEN.

When ordering please use the proper product designation as given on page 9. Sephadex ion exchangers are available in 100 g and 500 g packs and in bulk quantities of 5 kg or more.

LITERATURE SERVICE

As an aid to scientists, Pharmacia Fine Chemicals provides an extensive literature service. The Literature Reference Lists referred to in this booklet contain over 3,000 references pertaining to the use of Sephadex, Sephadex ion exchangers and other separation products. A detailed subject index facilitates information retrieval.

The Literature Reference Lists together with other specialized publications are available on request from Pharmacia Fine Chemicals or your local representative.

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June 1972-3 Appelbergs tryckeri

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